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Insecticidal potential of grapefruit and tangerine peel essential oils extracted by steam distillation

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Abstract This study focuses on extraction of essential oils from grapefruit and tangerine peels as an economical raw material for the production of insecticides. An equal mass each of grapefruit and tangerine peels were subjected to extraction via steam distillation process and the extracted essential oils were put to some physicochemical characterization. Some of the characterizations include specific gravity, acid value, and saponification value. Gas chromatography-Mass Spectroscopy analysis was also used to identify the chemical constituents of the oils and their percentage compositions. Tangerine peels showed highest yield (9ml) for 3hours compared to grapefruit peels (7ml) for the same extraction time. The specific gravities, acid values, and saponification values of tangerine and grapefruit peel oils were0.8429 and0.8540, 1.96 mg KOH/g oil and 15.99 mg KOH/g oil, and 182.7 mg KOH/g oil and 191.4 mg KOH/g oil respectively. From the Gas chromatography-Mass Spectroscopy analysis, 26 and 17 components were respectively identified in the tangerine and grapefruit peels essential oils with D-limonene being the dominant constituent of the oils (80.83% in tangerine and 92.64% in grapefruit peel oils). Based on availability, it was shown that grapefruit peel essential oil is an economical viable source for producing D-limonene. Exploitation of these essential oils will go a long way in reducing the infestation rate of malaria as they can be used to produce insecticides. It also showed that waste from peels which have huge impacts on the environment, could be converted into veritable source of industrial raw material with great potential for stimulating industrial and economic growth, employment generation and national wealth.

Keywords Grapefruit, Tangerine, Steam distillation, acid value, saponification, D-limonene

Introduction

Citrus, a major plant source of essential oils is one of the most important commercial fruit crops grown in all continents of the world [1]. They are naturally or hybrid cultivated, and the best known examples are, oranges, grapefruit, lemons, limes and tangerines. The citrus species are a potential source of variable oil which might be utilized for edible and other industrial application [2]. Citrus fruits have a rough, robust and bright (green to yellow) coloured skin. They are usually 4 to 30cm long and 4 to 20cm in diameter, with a leathery surrounding rind or skin known as epicarp (or flavedo) that cover the fruits and protect it from damages. Citrus fruits are notable for their fragrance, partly due to flavanoids and limonoids contained in the rind [3].

The grapefruit (*Citrus paradisi*) is a subtropical citrus tree known for its sour to semi-sweet fruit, an 18th century hybrid first bred in Barbados [4]. The grapefruit is a large citrus fruit related to the orange, lemon and pomelo. Grapefruits are usually categorized as white, pink or ruby. Grapefruits usually range in diameter from 10 to 15 centimetres, with some varieties featuring seeds while others are seedless. The wonderful flavour of a grapefruit is like paradise, just as its Latin name *Citrus paradisi*. It is juicy, tart and tangy with an underlying sweetness that weaves throughout. Tangerine (*Citrus tangerina*) is an orange-like citrus fruit that is much related to the mandarin orange (*Citrus reticulata*). The tangerine tree is smaller than the orange tree with slender branches, and deep-green leaves with pointed ends. It is flat, small compared to "Navel" or "Valencia" oranges. It has a loose skin (pericarp) which can be peeled rather easily. An average-sized fruit has 8-10 juicy segments (arils). The taste is considered less sour, as well as sweeter and stronger, than that of an orange [5].

Essential oils are the subtle, aromatic and volatile liquids extracted from the flowers, seeds, leaves, stems, bark and roots of herbs, bushes, shrubs and trees through various methods of extraction. They are also said to be used thousands of years ago to heal the sick making them the oldest form of medicine and cosmetics known to man. Essential oil from citrus is a large type of natural flavours and fragrances, which is popularly used in food industries, daily chemical product and health care field. Essential oils are complex mixtures of organic compounds that give characteristic odour and flavour to the plants. They can be found in the root such as that of the vetiver grass (*Vetiveria zizanioides*), in stems like that of peteribi wood (*Cordia trichotoma*) and incense, in leaves like in eucalyptus trees (*Eucalyptus citriodora*), citronella (*Cymbopogon nardus*), chinchilla (*Tagetes minuta*) and lemon grass (*Cymbopogon citratus*), in flowers like lavenders (*Lavandula officinalis*), in fruit like lemon, orange (Citrus spp.) and even in seeds as in the case of anise (*Pimpinella anisum*), coriander (*Coriandrum sativum*) and pepper (*Piper nigrum*), among others [6]. Citrus essential oils are rich in the powerful anti-oxidant d-limonene; they support the immune system and overall health while bringing about the sense of well-being, creativity and feeling of joy. Citrus essential oil contains insecticidal, antiseptic, anti-microbial, anti-bacterial, anti-fungal, astringent and anti-inflammatory properties. It could be used for cancer prevention, ulcer treatment, lowering cholesterol. It could also be used for weight loss, arthritis, and detoxification, skin toning and cellular regeneration.

In this part of the world, citrus peels are usually disposed in waste bins which have been part of the major causes of land pollution in the rural and urban areas with the rate at which citrus are being consumed. Also in industrial use, peels that are wasted after juicing, are usually eliminated by burning, which produces carbon dioxide and other greenhouse gases, or dumping into landfills, where the oil from rotting peels percolates into the soil, harming plant life. A fast need is arising in the curbing of pollution in the society which is caused by waste generated by the society itself. The citrus juice industry produces millions of tons of wet citrus peel waste annually. These peels are mostly dried and sold, often at a loss, as cattle feed to dispose of the waste residue. Profitability could be greatly improved if higher value products could be developed and produced from the peel waste. Extracting essential oils from these peels would improve the economic value as well as serve as a cheap raw material for health and industrial uses, which would not even stop its further use as a source of cattle feed.

Malaria is one of the major and most serious health problems facing the world today. It is endemic in Africa, particularly amongst pregnant women and children. More specifically, malaria is causing various problems in Nigeria. It is important to look at health problems like malaria that grossly affect mortality rate, as well as the economy of a developing country, such as Nigeria. Nigeria has a population of about 168.8 million people where a large percentage of it lives in extreme poverty in rural areas, without access to treated mosquito nets and adequate healthcare. Exploitation of these essential oils will go a long way in reducing the infestation rate of malaria as D-limonene can be used as the basic raw material in the production of mosquito repellents such as insecticides. This paper therefore investigated the insecticidal potentials of grapefruit and tangerine peel essential oils extracted by steam distillation.

Materials and Methods

Extraction via steam distillation

The grapefruits and tangerines used were bought from Ketu market in Lagos state, Nigeria, and were taken to Chemical engineering Laboratory, University of Port Harcourt, Rivers state, Nigeria. All the chemicals used in this work were of reagent grade. The grapefruits and tangerines were washed with de-ionized water, thin peeled and then cut into small bits. The grapefruit and tangerine peels were weighed separately using an electronic weighing balance to obtain exactly 400grams each. The peels were then pureed using a blender. This procedure is based on the extraction of oil from grapefruit peels since the same applies for tangerine peels.

The pureed grapefruit peel was carefully transferred into a 1000ml flat bottom flask. 600ml of de-ionized water was measured using a 1000ml measuring cylinder and which was then added to the 1000ml flat bottom flask that is serving as the distilling flask. Boiling chips was then added to the distilling flask to ensure that boiling occurs calmly without bumping. The condenser water was turned on and the distilling flask was properly seals. Heating of the distilling flask was then slowly commenced and the heat source was adjusted so that the distilling rate equals about 20 drops per minutes. As the mixture boils and distils, the water level in the distilling flask drops, de-ionized water was then added via the separation funnel in the set-up to keep the water level at the required level preventing the water level from going too low. It is very important to keep the water level at an operational level because of the high concentration of sugar in the peels. If the Water level gets too low, the sugar will caramelize and burn. It is also of great importance to add small amounts of water so that the temperature doesn't drop drastically. The experiment was conducted for the following times 60, 90, 120, 150, and 180 minutes, in each case, the distillate was collected and transferred into a 250ml separating funnel. 20ml of diethyl ether was added to the distillate in the separating funnel to extract the oil. It was ensured that the hot plate was off and there was no flame source during the use of diethyl ether because diethyl ether is very, very flammable. The water layer in the separating funnel was drained off and the ether layer into a small collection flask. The ether was then dried with a little anhydrous magnesium sulfate. This is required to dry off the trace amounts of water in the ether. The anhydrous magnesium sulfate will absorb the water and produce solid magnesium sulfatedecahydrate:

$$MgSO_4(s) + 10H_2O \rightarrow MgSO_4(s) \bullet 10H_2O\ (s)$$

The medium was allowed to dry for 15 minutes. It was turned into another flask and then kept in a fume cupboard for 12 hours to allow all the diethyl ether to vaporize. The volume and mass of the oil extracted was then measured and recorded.

Sensory analysis of the essential oils

The sensory analysis carried out was to find out some essential properties of the oil without the aid of any equipment. It was done using the sense organs of sight, smell and touch.

Physicochemical analyses of the essential oils

Solubility

4 drops of grapefruit essential oil was added to the test tube containing 8 drops of water. The test tube was then stirred thoroughly with a laboratory glass stirring rod. The mixture was allowed to stay for 5 minutes. Two separate liquid phases were observed with the water being at the bottom. The pH of the water was measured to check for the solubility of the essential oil in water. The above experiment was also carried out on tangerine essential oil.

Boiling point

5ml of grapefruit essential oil is placed a small test tube. A capillary tube is placed inside the test tube with the closed end upward. The test tube is clamped to a ring stand, and a thermometer was attached to it. A 250ml beaker was half filled with water, and placed on the hot plate. The test tube and thermometer combination were carefully lowered into the beaker of water so that the test tube is immersed half way in the water. The medium was heated slowly with the hot plate. As the oil approaches its boiling point, few bubbles were observed flowing out of the end of the capillary tube. When steady streams of bubbles were observed, the hot plate was turned off and the contents of the test tube were allowed to cool. As the contents of the test tube cooled, the capillary tube was observed carefully. The temperature at which the oil began to flow into the capillary tube was recorded. The above experiment was repeated for tangerine essential oil.

Specific gravity

A clean and dry density bottle of 25ml capacity was weighed to give W_0. It was filled with water and reweighed to give (W_1). The water was drained and bottle was clean to ensure that it is totally free of water. It was then filled with grapefruit essential oil and weighed to give (W_2). The specific gravity was then determined from the following equation. The above experiment was carried out again using tangerine essential oil.

$$Specific\ gravity = \frac{W_2 - W_0}{W_1 - W_0}$$

Refractive index

Abbe's refractometer was used for the determination of refractive indices of the oils. There refractive index is denoted by n_D^{25}" where n is the refractive indexat25°Ctakenwithsodiumlight (D-line). The refract meter was calibrated with distilled water (RI of 1.3330 at 20°C/68°F and 1.3325 at 25°C/76°F) which has refractive index of$n_D^{29.5}$=1.3315. The glass prism was then cleaned by wiping with cotton pad moistened with acetone and let air dry. After this, a drop of grape fruit essential oil was placed between the prisms of refracts to meter and allowed time for temperature equilibrium between the instrument and the sample. The telescope was rotated to bring the border line of total refractiontothejunctionofcross-wireinthetelescope.Therefractiveindexwas recorded at room temperature. The above experiment was repeated for tangerine essential oil.

Acid value

10g of the grapefruit essential oil was placed in a dried conical flask**.** 50ml of ethyl alcohol was added into the conical flask and 3 drops of phenolphthalein was added to it. The solution was titrated against 0.1N KOH until pink colour appeared (end point)**.** The volume of KOH used in the titration was recorded. The above process was repeated with tangerine essential oil. The acid value was calculated with the equation:

AV (mg KOH/g sample) = $\frac{56.1\ x\ Vx\ N}{m}$

Where, V – Titre volume of standard potassium hydroxide solution (mL);

N – Normality of the potassium hydroxide solution, m – Mass of oil sample (g)

Saponification value

2g of grapefruit essential oil was weighed into a 250 ml conical flask, to which 50ml 0.5 M alcoholic KOH was added. The content was constantly stirred for 1 hour followed by reflux. 3 drops of phenolphthalein indicator was added and titrated with 0.5 M HCl until the pink coloration disappeared. The procedure was repeated for tangerine essential oil. The saponification value was calculated with the equation:

$$SV\ (mg\ KOH/g\ sample) = \frac{[(S-B) \times M \times 56.1]}{Sample\ \ weight}$$

Where, S = sample titre value (mL), B = blank titre value (mL), M = molarity of the HCl

GC-MS analysis of the essential oils

The essential oils were analysed for their chemical composition by GC-MS analysis. An Agilent 7890A gas chromatograph was used. Nitrogen as carrier gas, split ratio 1:50, electrical energy 70 eV, ionization temperature 250°C, the column temperature was programmed from 70°C (2min) to 250°C (15 min) at a rate of 10°C/ min. The injector and detector temperatures were 200°C and 250°C respectively. The chemical constituents were identified by their retention time.

Insecticide preparation

The camphor was crushed into powder. 60ml of kerosene and 20ml of grapefruit peel essential oil was mixed in the 250ml beaker, and the crushed camphor was added gradually to the homogeneous mixture of the grapefruit peel essential oil and the kerosene and stirred with the stirrer until the mixture becomes saturated with the camphor. The mixture was then decanted and stored in an air tight container.

Results and Discussion

Steam distillation

The steam distillation method of extraction used in this work proved to be a success. From the experiment, it was discovered that the rate of extraction of the essential oil was higher during the first hour as shown in Table 1 and Figure 1 for both grapefruit peel and tangerine peels.

Table 1: Volume of oil extracted from the peels with respect to time

S/N	Heating time (min)	Volume extracted (ml)	
		Grapefruit	Tangerine
1	60	4	4.5
2	90	5	6
3	120	6	7.5
4	150	6.5	8.5
5	180	7	9

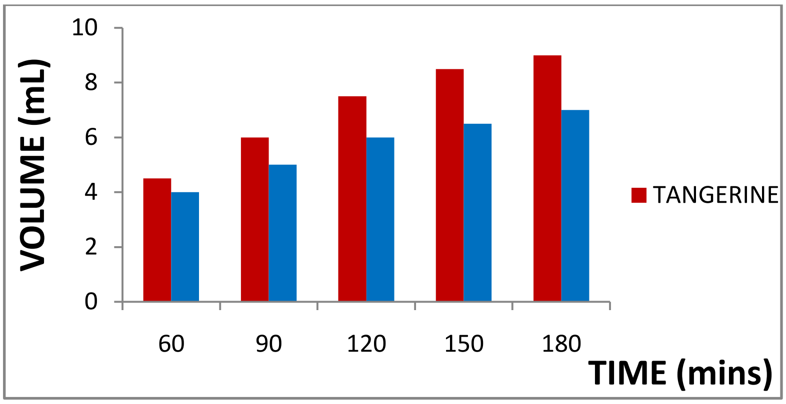

Figure 1: A bar chart showing the volumes of grapefruit and tangerine extracted at different times

It was noticed that more oil was extracted from tangerine peels compared to the ones extracted from grapefruit peels. The observations are in agreement with the findings of Kamal et al. (2011) [7] who reported that among the citrus species tested, *C. Sinensis* exhibited the maximum oil yield (0.24-1.07%) followed by *C. reticulata* (0.30-0.50%) and C. paradisi (0.20-0.40%). It is even possible that a consistent and better heat source other than the stove used in this work for the steam distillation would have improved the extraction success rate. Also, Gamarra et al. (2006) [8] stated that the quality of essential oil depends on different factors; among them are the chemotype and biotype of the plant, the climatic conditions as well as the extractive process. For as little as 400g of grapefruit and tangerine peels to yield as much as 7ml and 9ml of oils respectively, it means for large amounts of the waste peels during tangerine and grapefruit season, reasonable amounts of essential oils would be produced.

Sensory analyses results of the essential oils

From the sensory analysis tests carried out on the oils with the aid of sense organs, it was obtained that both the oils had light yellow coloration, pleasant citrus-like smell and very light at hand show signs of low density than water. These observations are in agreement with the findings of Javed S. et al (2014) [9] who reported that among the citrus species tested, grapefruit peel and tangerine peel oils have light yellow coloration and pleasant smells.

Physicochemical analyses results of the oils

The physicochemical properties of grapefruit peel and tangerine peel essential oils are tabulated in Table 2. The specific gravity and refractive indices present in this work were between 0.842-0.854 and 1.473-1.476 respectively. These results were in line with preceding work on essential oil of citrus species [9].

Table 2: Physicochemical analyses results of the essential oils

Property	Results Obtained	
	Grapefruit	Tangerine
Specific gravity	0.8540	0.8429
Refractive index	1.4760	1.4730
Boiling point	172°C	179°C
Solubility	Insoluble in water	Insoluble in water
Acid value	15.99 mg KOH/g oil	1.96 mg KOH/g oil
Saponification value	191.4 mg KOH/g oil	182.7 mg KOH/g oil

Chemical composition of the essential oils

The results obtained from GC-MS analysis for the grapefruit peel and tangerine peel essential oils were presented in Table 3. The GC-MS chromatogram for tangerine peel and grapefruit peel essential oils are shown in Figures 2 and 3 respectively. From the results obtained, grapefruit peel essential oil has 17 components and tangerine peel essential oil has 26 components. These results are in conformity with the previous findings on citrus peels. Terpenes and oxygenated compounds such as limonene, γ-terpinene, β-pinene, α-pinene, myrcene, valencene, linalool, octanal, decanal, and butyle butyrate has been found as the major constituents through GC separation [10-11].

Table 3: Components and percentage composition in grapefruit peel essential oil

S/n	Component	Composition (%)	
		Grapefruit	Tangerine
1	Limonene	92.64	80.831
2	ρ-cymene	1.63	0.784
3	Borneol	0.59	0.157
4	Trans-β-ocimene	0.30	0.284
5	α-terpinene	0.40	1.233
6	ϒ-terpinene	0.23	0.264
7	1,8-Cineole	-	0.303
8	Terpine-4-ol	1.07	0.191
9	Linalool	0.24	1.372
10	Octanal	-	1.099
11	m-cymene	0.25	1.495
12	Citronellal	0.23	0.200
13	Camphene	0.19	0.170
14	β-phellandrene	-	0.260
15	Salicylaldehyde	-	0.365
16	α-phellandrene	-	0.281
17	1-Nonanol	0.18	-
[illegible]	[illegible]	[illegible]	[illegible]
19	Trans-sabinol	0.14	0.161
20	Trans-linalool	0.22	5.919

21	2-Nonanone	-	0.329
22	Trans-limonene	-	0.553
23	Cis-β-terpineol	0.11	-
24	Benzene acetaldehyde	0.16	-
25	Nerol oxide	-	0.161
26	Cis-linalool oxide	0.1	1.161
27	3-Hexenal	-	0.160
28	Cis-sabinol	-	0.118
29	Camphor	-	0.154

The predominant component of the essential oils is D-limonene as it has a percentage composition of 92.64% in grapefruit peel essential oil and a composition of 80.83% in tangerine peel essential oil. The minor components were mostly less than 1% of the oil composition. Most of these components as well as D-limonene being the predominant have been previously reported in a previous study citrus peel oils [9].

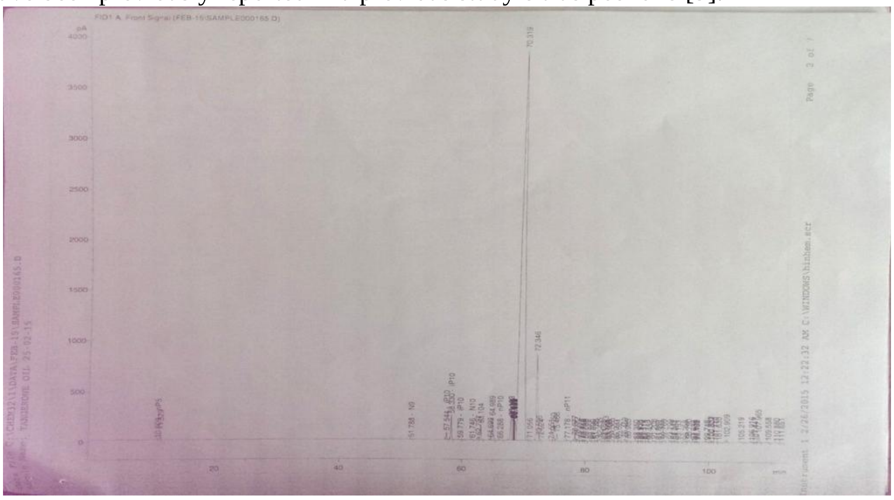

Figure 2: Chromatograph of tangerine peel essential oil

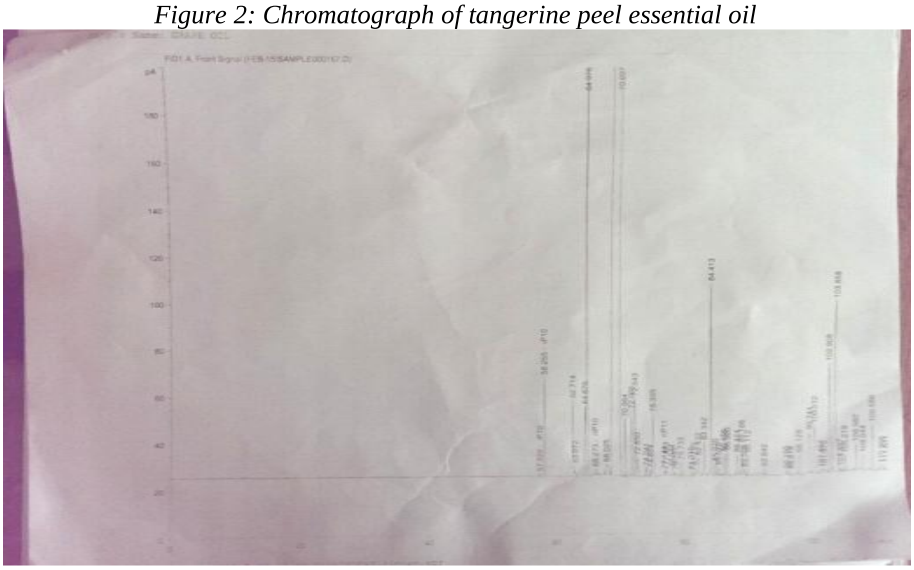

Figure 3: Chromatograph of grapefruit peel essential oil

However, the percentage composition of D-limonene present in grapefruit peel and tangerine peel essential oils in this study did not agree with the ones [9]. This discrepancy could possibly be as a result of the fact that quality of essential oil depends on different factors; among them are the chemo-type and biotype of the plant, the climatic conditions as well as the extractive process [8]. It could also by the plant health and harvest time.

Insecticide action against insects

The insecticide produced was kept in an air tight container to avoid oxidation of the contents. It was tested on some insects (cockroach and black ants) and found to be effective as it was able to kill the insects within a space of 2 minutes because of the high content of limonene in the grapefruit peel essential oil used as part of the raw materials used in producing it.

Conclusion

From the results obtained from this study, it is shown that grapefruit peels contains less oil as compared to tangerine peels but has a higher limonene content than tangerine peel. Thus, it can be concluded that grapefruit peel is more economical viable than tangerine peels because of its higher rate of limonene and availability. This study has also shown that the oil extract of grape peels from Nigeria contains more limonene as compared to other regions. Thus, it may be considered as a cheap source of raw material in the production of insecticide to fight the growth rate of malaria in the country. Also, this study has shown that waste from peels which have huge impacts on the environment, could be converted into veritable source of industrial raw material with great potential for stimulating industrial and economic growth, employment generation and national wealth.

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